Pedobiologia 35, 179–184 (1991)
Gustav Fischer Verlag Jena

Zoological Laboratory, Institute for Zoology and Zoophysiology University of Aarhus, Denmark

The relationship between temperature and cocoon incubation time for some Lumbricid earthworm species

M. HOLMSTRUP, I. K. ØSTERGAARD, A. NIELSEN and B. T. HANSEN

With 2 figures

(Accepted: 90-10-28)

1. Introduction

In recent years much research has established the importance of earthworms for soil fertility in arable land. This has resulted in many investigations aimed at revealing how agricultural practices, including the use of fertilizers and pesticides, influence the dynamics of earthworm populations. In such investigations the estimation of cocoon numbers, in addition to numbers of adult and juvenile worms, can contribute valuable information on population dynamics and give an estimate of the reproductive rate of the earthworms under field conditions (BOSTRÖM, 1988).

To make full use of cocoon data, however, knowledge of the incubation time of earthworm cocoons, and how this is controlled by environmental factors, is needed. This study seeks to evaluate the influence of temperature on the incubation time of the cocoons of some earthworm species important in agricultural soils. In addition, an earthworm species common in woodland is investigated.

2. Materials and methods

Incubation times were measured for the species *Aporrectodea caliginosa* (SAV.), *Aporrectodea rosea* (SAV.), *Allolobophora chlorotica* (SAV.), and *Dendrobaena octaedra* (SAV.), cocoons being obtained from laboratory cultures.

Earthworms were held in 1-litre plastic pots with lids, small holes in the lids allowing ventilation. Each species was held separately with 4–5 adult worms per pot with the exception of *D. octaedra*, for which the number was 8–10 adult worms. All pots were kept in darkness at 15 °C (±1 °C).

The culture substrate was a loamy soil with a moisture content of 16–18% (based on hygromass) and the earthworms were fed on cowdung mixed with soil in equal parts per volume. Prior to use, the soil in the cowdung mixture was wet sieved through a 2 mm mesh to hinder foreign cocoons being inadvertently introduced to the cultures. Also before use, the cowdung and soil used as culture substrate was dried at 80 °C to kill any worms, cocoons or predators present.

In order to obtain a large number of cocoons the worms were allowed to breed for a period of 21 days. After this the contents of the pots were wet sieved through a 1 mm sieve. The cocoons of each species were placed in petri dishes on filter paper wetted with tap water. The lids of the petri dishes had small holes to ensure that oxygen conditions were optimal for cocoon development.

Four experiments were carried out in which cocoons, placed in petri dishes, were allowed to develop and hatch at constant temperatures of 5, 10, 15 and 20 °C (±1 °C), respectively. However, at 5 °C the embryos developed only slowly and so, for practical reasons, after ca. 200 days the cocoons were transferred to 20 °C where development of non-hatched cocoons was completed.

The petri dishes were inspected twice weekly and the number of hatched cocoons was noted.

If necessary, tap water was added to the filter paper to maintain optimal moisture conditions. If fungi had developed on the filter paper the cocoons were rinsed in tap water and the filter paper was changed.

The experiments continued until all viable cocoons had hatched.

3. Calculations

The following involves two different values, the observed incubation time in the experiments (I_{obs}), and corrected incubation time (I_{cor}).

For calculation of the average observed incubation time (I_{obs}) in each experiment, it is assumed that the worms in the cultures have produced cocoons regularly during the 21-day breeding period, and thus the cocoons have an average age of 10.5 days by the time of the wet sieving. Similarly, it is assumed that the hatching of a cocoon, noted at a given inspection date, has taken place half way between this inspection date and the previous one.

The calculations described in the following paragraphs are all based on mean values derived from the total number of cocoons hatched in each experimental series.

The observed incubation times at 5, 10 and 20 °C are not the correct incubation time (I_{cor}) as cocoons in all four experiments had developed at 15 °C in the period before wet sieving. Only for cocoons held at 15 °C is the correct incubation time ($I_{15\,cor}$) equal to the observed incubation time ($I_{15\,obs}$).

By using the rate of development at 15 °C ($I^{-1}_{15\,cor}$), the percentage of development in the 10.5 days before wet sieving, PD_{15}, can be calculated as:

$$PD_{15} = (I^{-1}_{15\,cor} \times 10.5 \text{ days} \times 100)\% \,. \tag{3.1}$$

This means that only $(100 - PD_{15})\%$ of the full development of the embryo took place at 10 and 20 °C. The actual number of days where the embryo developed at 10 and 20 °C is $(I_{10\,obs} - 10.5)$ days and $(I_{20\,obs} - 10.5)$ days, respectively. Thus, the correct incubation time at 10 °C ($I_{10\,cor}$) and 20 °C ($I_{20\,cor}$) can be calculated as:

$$I_{10\,cor} = \frac{100}{(100 - PD_{15})} \times (I_{10\,obs} - 10.5) \text{ days} \,, \tag{3.2}$$

$$I_{20\,cor} = \frac{100}{(100 - PD_{15})} \times (I_{20\,obs} - 10.5) \text{ days} \,. \tag{3.3}$$

When calculating the correct incubation time at 5 °C ($I_{5\,cor}$) both the development of the cocoons in the period before wet sieving and the development of the cocoons after having been transferred to 20 °C must be taken into account. The average percentage of development which has taken place at 20 °C in the 5 °C experiment is denoted PD_{20} and is calculated as:

$$PD_{20} = \frac{\text{average number of days at 20 °C needed for a cocoon to hatch}}{I_{20\,cor}} \times 100\% \,. \tag{3.4}$$

The average number of days at 20 °C needed for a cocoon to hatch is the mean value for all cocoons in the 5 °C experiment (if a cocoon hatched at 5 °C it has been maintained for 0 days at 20 °C).

The correct incubation time at 5 °C is then calculated as:

$$I_{5\,cor} = \frac{100}{(100 - (PD_{15} + PD_{20}))} \times (\text{average number of days maintained at 5 °C}) \text{ days} \,. \tag{3.5}$$

The average number of days that cocoons are maintained at 5 °C is the mean value for all cocoons in the experiment, both those that hatched at 5 °C and those that hatched after having been transferred to 20 °C.

4. Results

Figure 1 shows observed hatching of the cocoons in the four experiments. For all species, the period during which hatching takes place increase as incubation temperature decreases. In the 5 °C experiment cocoons hatch shortly after having been transferred to 20 °C. Because of a technical mistake no data in the 5 °C experiment for *A. rosea* were obtained.

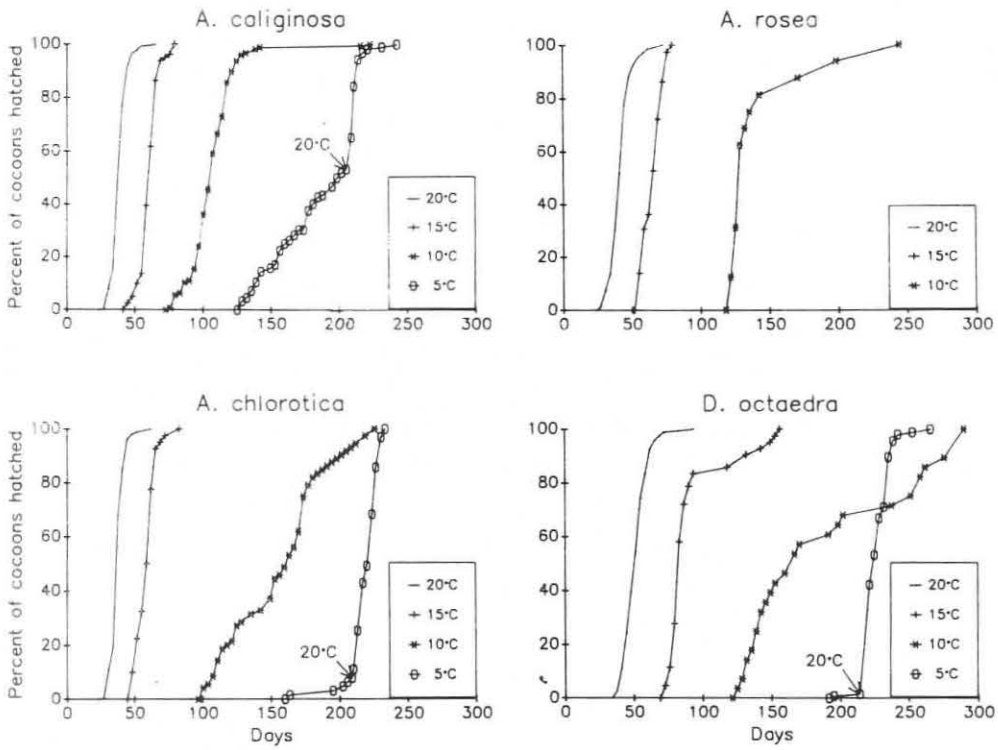

Fig. 1. Cumulative percentage of hatching of cocoons incubated at 5, 10, 15 and 20 °C. Arrows indicate the time at which cocoons in the 5 °C experiment were transferred to 20 °C.

Average incubation times (I_{cor}) for cocoons at 20, 15, 10 and 5 °C are presented in table 1. Incubation times are similar for *A. caliginosa*, *A. chlorotica* and *A. rosea* at 20 °C, namely 34 – 38 days, and at 15 °C, namely 59 – 66 days. *D. octaedra* cocoons have a longer incubation period: 47 days at 20 °C and 92 days at 15 °C. At 10 °C, *A. caliginosa* has the shortest incubation time followed by *A. rosea*, *A. chlorotica* and *D. octaedra*. At 5 °C, *A. chlorotica* and *D. octaedra* cocoons develop in ca. 400 days, whereas *A. caliginosa* cocoons develop within a much shorter time, this being ca. 230 days.

Table 1. Average incubation time (I_{cor}) in days for cocoons maintained at 5, 10, 15 and 20 °C.

Species	5 °C	10 °C	15 °C	20 °C
A. caliginosa	234 (n = 151)	119 (n = 139)	62 (n = 81)	36 (n = 489)
A. rosea		157 (n = 16)	66 (n = 36)	38 (n = 66)
A. chlorotica	418 (n = 63)	178 (n = 69)	59 (n = 40)	34 (n = 222)
D. octaedra	402 (n = 118)	203 (n = 28)	92 (n = 43)	47 (n = 219)

Note: n is the number of observations in each experimental series.

Figure 2 shows the relationship between rate of embryonic development and temperature. There is a clear linear relationship between $\log_{10}$ rate of development and temperature, indicating that rate of development, V, is dependent on temperature, t, in an exponential manner: $V = aK^t$, where a and K are species dependent constants.

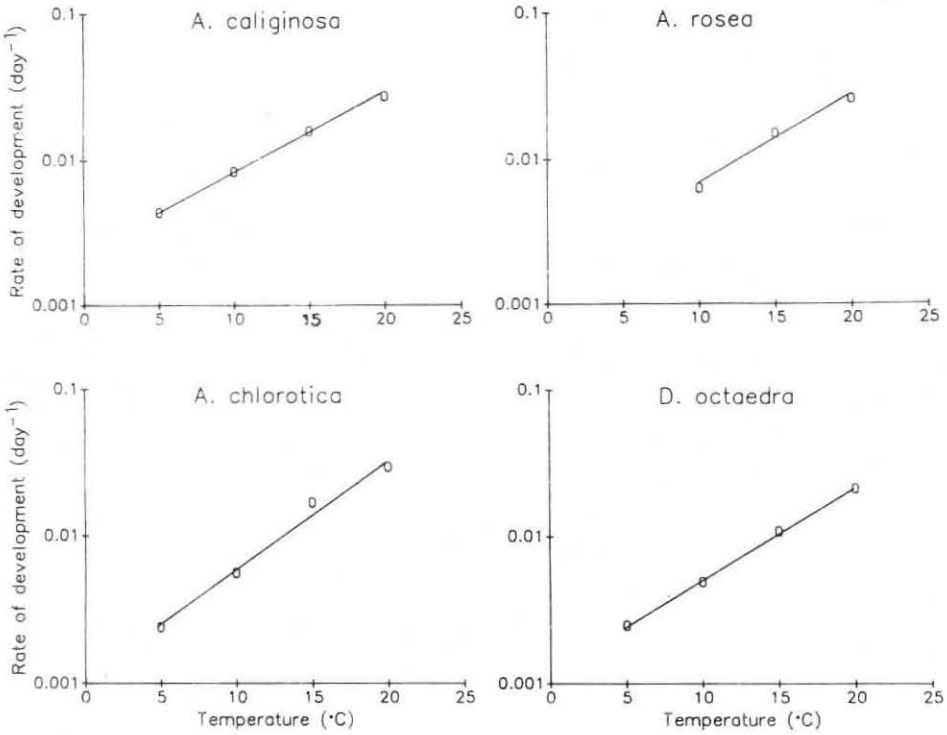

Fig. 2. Rate of development (I_{cor}^{-1}) plotted on a logarithmic y-axis against temperature. By linear regression the formulas for rate of development, V, are found. *A. caliginosa*: $V = 0.0024\ 1.13^t$, $r^2 = 0.9882$; *A. rosea*: $V = 0.0016 \cdot 1.15^t$, $r^2 = 0.9839$; *A. chlorotica*: $V = 0.0011 \cdot 1.19^t$, $r^2 = 0.9855$; *D. octaedra*: $V = 0.0012 \cdot 1.16^t$, $r^2 = 0.9990$.

5. Discussion

The estimated incubation times of *A. chlorotica* and *A. caliginosa* cocoons at 20 and 15 °C agree well with the observations of GERARD (1967), who also noted that the incubation time of *A. caliginosa* cocoons was slightly longer than that of *A. chlorotica* for both these temperatures. Incubation time at 10 °C for *A. chlorotica* (178 days) is somewhat different from the value found by GERARD (112 days), perhaps due to methodological differences.

Other detailed investigations regarding incubation time of earthworm cocoons exist only sporadically in the literature. GRAFF (1953) noted that *A. caliginosa* cocoons hatched in 65 days at 12 °C and BOSTRÖM (1988) found incubation time for the same species to be 70–84 days at 15 °C. GRAFF (1953) noted that incubation time for *A. rosea* was 60 days at 12 °C. The results of the present investigation are in fairly good agreement with the observations of these authors.

SIMS & GERARD (1985) claimed that embryonic development of *A. chlorotica* and *A. caliginosa* was arrested immediately when cocoons were exposed to temperatures of 5 °C or below, but this was not found in the present investigation. On the contrary, embryonic development took place in *A. caliginosa*, *A. chlorotica* and *D. octaedra* at this temperature. *A. caliginosa* hatched readily at 5 °C and some hatching of *A. chlorotica* and *D. octaedra* cocoons began after ca. 200 days (figure 1).

As shown in figure 2, rate of development decreases curvilinearly with decreasing temperature in the investigated temperature range, but the lower temperature thresholds

for development or hatching were not determined. Apparently, *A. caliginosa* cocoons develop and hatch at lower temperatures than do *A. chlorotica* and *D. octaedra* cocoons (figures 1 and 2).

RUNDGREN (1977) proposed the existence of a lower threshold temperature below which hatching does not occur. This proposal was based on a field investigation in which newly emerged *A. caliginosa* and *A. rosea* juveniles were not observed in spring until soil temperature had reached 4–6 °C. Similarly, newly emerged *Lumbricus terrestris* were not found in samples before soil temperature in spring had reached 8 °C. The increasing temperature in spring may therefore have a "trigger" effect on hatching. This is illustrated for the species investigated in the 5 °C experiment where hatching suddenly increased when the cocoons were transferred to 20 °C (figure 1). These observations imply that embryonic development can take place even though the temperature is below the threshold for hatching.

The ability of the embryo to develop at low temperatures, even at a slow rate, means that cocoons deposited in autumn or late summer have a better chance of being ready to hatch in spring as soon as environmental conditions, such as temperature and food availability, are favourable. In a similar way the threshold temperature for hatching should be regarded as an adaptation to the particular habitat conditions in which the species is living.

The rather long incubation period found for *D. octaedra* could be one reason why this species has a more northern distribution than do the three other species investigated (*cf.* STÖP-BOWITZ, 1969). *D. octaedra* is adapted to a cold climate, the cocoons being very tolerant to frost, whereas cocoons of *A. caliginosa*, *A. chlorotica* and *A. rosea* are not (HOLMSTRUP *et al.*, 1990). On the other hand, *A. caliginosa*, *A. chlorotica* and *A. rosea* are well adapted to a warmer habitat and a long season of activity and growth by having a short incubation period.

6. Acknowledgements

We would like to thank Dr. OLE CHRISTENSEN and Dr. JANICE G. MATHER for valuable comments on the manuscript.

7. References

BOSTRÖM, U., 1988. Ecology of earthworms in arable land. Population dynamics and activity in four cropping systems. Swedish University of Agricultural Sciences. Department of Ecology and Environmental Research. Report no. 34.

GERARD, B. M., 1967. Factors affecting earthworms in pastures. J. Anim. Ecol. **36**, 235–252.

GRAFF, O., 1953. Die Regenwürmer Deutschlands. Schrift. Forsch. Land. Braunschweig Völkenrode **7**.

HOLMSTRUP, M., B. T. HANSEN, A. NIELSEN & I. K. ØSTERGAARD, 1990. Frost tolerance of lumbricid earthworm cocoons. Pedobiologia **34**, 6, 361–366.

RUNDGREN, S., 1977. Seasonality of emergence in lumbricids in southern Sweden. Oikos **28** (1), 49–56.

SIMS, R. W., & B. M. GERARD, 1985. Earthworms. Synopses of the British fauna no. 31. London.

STÖP-BOWITZ, C., 1969. A contribution to our knowledge of the systematics and zoogeography of norwegian earthworms (Annelida Oligochaeta: Lumbricidae). Nytt Mag. Zool. **17**, 169–280.

Synposis: *Original scientific paper*

HOLMSTRUP, M., I. ØSTERGAARD, A. NIELSEN & B. T. HANSEN, 1991. The relationship between temperature and cocoon incubation time for some lumbricid earthworm species. Pedobiologia **35**, 179–184.

The duration of incubation time in relation to ambient temperature was measured for cocoons of four lumbricid earthworm species. Cocoons were obtained from laboratory cultures of *Aporrectodea caliginosa* (SAV.), *Aporrectodea rosea* (SAV.), *Allolobophora chlorotica* (SAV.) and *Dendrobaena octaedra* (SAV.). The cocoons were incubated at 5, 10, 15 and 20 °C at optimum moisture conditions.

Incubation time increased greatly with decreasing temperature but embryonic development took place at all four experimental temperatures. *A. caliginosa*, *A. rosea* and *A. chlorotica* cocoons developed within 34–38 days at 20 °C and within 59–66 days at 15 °C. *D. octaedra* cocoons developed within 47 days at 20 °C and 92 days at 15 °C. At 10 °C, *A. caliginosa* had the shortest incubation time followed by *A. rosea*, *A. chlorotica* and *D. octaedra*. At 5 °C, *A. chlorotica* and *D. octaedra* developed in ca. 400 days whereas *A. caliginosa* needed only ca. 230 days.

In the present investigation it is suggested that a lower threshold temperature for hatching exists, but that embryonic development can still take place even though temperature is below this threshold. The ability of the embryo to develop at low temperatures should be regarded as an adaptation to the particular habitat, in which the species is living, making it possible for the juvenile to emerge as soon as environmental conditions are favourable.

Key words: Earthworms, cocoons, incubation time, temperature, hatching.

Address of the corresponding author: Dr. MARTIN HOLMSTRUP, Zoologisk Laboratorium, Institut for Zoologi og Zoofysiologi, Aarhus Universitet (Zoological Laboratory, Institute for Zoology and Zoophysiology, University of Aarhus) Universitetsparken, Bygning 135, Aarhus C, DK-8000, Danmark.